METHANOL, A POTENTIAL FEEDSTOCK FOR BIOTECHNOLOGICAL PROCESSES

DIJKHUIZEN, L; HANSEN, T A; HARDER, W

Published in:
Trends in Biotechnology

DOI:
10.1016/0167-7799(85)90026-5

Publication date:
1985

Methanol, a potential feedstock for biotechnological processes

Lubbert Dijkhuizen, Theo A. Hansen and Wim Harder

A wide variety of bacteria and yeasts is able to grow in inexpensive synthetic media with methanol as the sole or major source of carbon and energy. This is due to the presence of a few unique enzymes which enable these organisms to generate metabolic energy and synthesize cell constituents from this one-carbon substrate. In the chemical industry there is currently much interest in the production of fuels and chemicals from methanol. As a feedstock for industrial fermentations methanol is also attractive because of its low cost, ease of handling and abundant availability. In many countries methanol-utilizing microbes are being studied and their potential utility in biotechnological processes is explored. These studies are aimed at making use of their characteristic properties, exploiting known organisms and new strains for improving existing processes and developing novel products.

Methanol is a one-carbon compound which can be made from synthesis gas, a mixture of carbon monoxide and hydrogen. Most synthesis gas is currently manufactured by steam reforming of natural gas, but alternative sources are coal and (residual) oil. In the chemical industry there is increasing interest in methanol both as an alternative source for the production of chemicals commonly derived from ethylene, and as a fuel[1]. This interest is based on economic considerations (i.e. the low price of methanol (6 c/lb) and the billion-gal/yr surplus capacity for its production in the world) and on technological innovations which allow the production of such important industrial chemicals as vinylacetate, ethylene glycol and ethanol via new routes from methanol as a feedstock[1]. The significance of these new technologies is that they provide a direct route from coal to derivative industrial chemicals, thereby circumventing dependence on crude oil.

As an alternative to chemical processes, methanol and other one-carbon compounds can also be converted into a variety of products by biological processes. These processes use microorganisms which can grow on one-carbon compounds as the sole or major source of carbon and energy. These organisms possess, in addition to the metabolic machinery common to most living cells, a number of unique enzymes that enable them to generate energy from methanol and synthesize all cell constituents required for growth and reproduction from this C_1 carbon source. This means that they are able to synthesize from methanol a variety of carbon compounds (similar to that produced by organisms currently used in the fermentation industry) from more complex and more expensive carbon sources. It is this ability of methylotrophs that is the main reason for the current interest in the application of these organisms in biotechnological processes. Initially this interest mainly focused on the production of single-cell protein (SCP) from methanol, but more recently it has turned towards the production of useful chemicals. Compared to chemical processes, the use of methylotrophs in bioprocesses has the added advantage that multi-step reactions for the synthesis of complex materials under ambient process conditions are feasible.

In this review we will concentrate on potential opportunities offered by methanol-using organisms. For information on methane-utilizing or methane-generating organisms the reader is referred to recent reviews[2-4]. For the present discussion it may suffice to briefly mention that methanol has distinct advantages over methane as a feedstock of biotechnological processes. This is not only due to physical and chemical considerations (i.e. methanol is completely miscible with water, is easy to store, to transport and to handle), but also to the properties of the pertinent organisms. Compared to methane, aerobic processes based on methanol usually show higher rates and higher yields of formation of carbon products and have a lower oxygen demand. Furthermore, methanol is used by a number of fermentative anaerobes, whereas methane is not.

As outlined below, the metabolic pathways of methanol metabolism are largely known and this permits an analysis of the potential utility of methylotrophs. From this analysis a number of questions arise which point the way to future research on these interesting organisms.

Physiology of methylotrophs

The metabolism of methylotrophs has been intensively researched during the last two decades and this has resulted in the accumulation of a wealth of information in the recent literature[5-17]. A general picture of methanol metabolism is drawn in this section and, when relevant to possible biotechnological applications, unique features of methylotrophic microorganisms are emphasized.

Aerobic methanol-utilizing bacteria and yeasts

Over the years it has become clear that methylotrophs possess special pathways both for the generation of energy and for carbon assimilation. Oxidation of methanol proceeds via formaldehyde to CO_2 (Fig. 1). In bacteria this involves a methanol dehydrogenase, whose prosthetic group was originally identified in *Hyphomicrobium* X as pyrroloquinoline quinone.

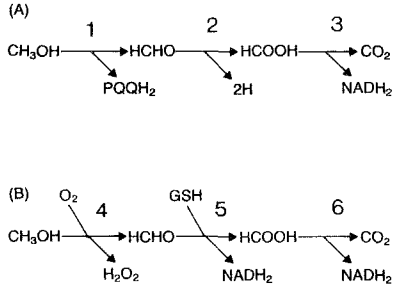

Fig. 1. Pathways for methanol oxidation in bacteria (A) and yeasts (B). 1: methanol dehydrogenase; 2: formaldehyde dehydrogenase; 3: formate dehydrogenase; 4: alcohol oxidase; 5: glutathione-dependent formaldehyde dehydrogenase; 6: formate dehydrogenase (for details: see text).

Lubbert Dijkhuizen, Theo A. Hansen and Wim Harder are at the Department of Microbiology, University of Groningen, Kerklaan 30, 9751 NN Haren, The Netherlands.

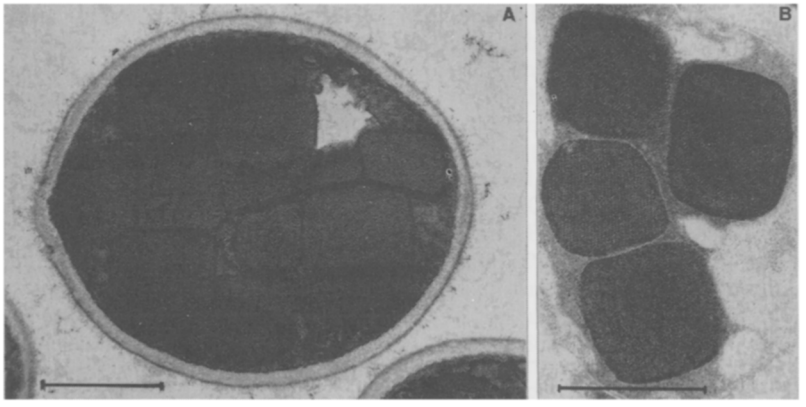

Fig. 2. Cells of Hansenula polymorpha, *grown in a methanol-limited chemostat at a dilution rate of $D = 0.05\ h^{-1}$, closely packed with several cubically shaped peroxisomes (A; fixation $KMnO_4$). Alcohol oxidase activity in the organelles is demonstrated after incubation of glutaraldehyde-fixed spheroplasts of these cells with $CeCl_3$ and methanol. Note that the crystalline substructure of the peroxisomal matrix is well preserved under these conditions (B; fixation glutaraldehyde-OsO_4). The markers represent 1 μm.* Courtesy of M. Veenhuis.

This novel coenzyme is also present in several other pyridine nucleotide-independent dehydrogenases in both methylotrophs and non-methylotrophs. In all methanol-utilizing yeast species investigated so far the conversion of methanol into formaldehyde (and H_2O_2) is catalysed by alcohol oxidase. This enzyme has been purified from *Hansenula polymorpha* and *Candida boidinii* amongst others and consists of eight identical subunits each containing one molecule of FAD. In these two organisms, alcohol oxidase and catalase are exclusively located in microbodies (peroxisomes; see Fig. 2) and high levels of alcohol oxidase (up to 30% of the total soluble protein) are synthesized under methanol-limiting conditions.

The formaldehyde produced from methanol can be further oxidized in several ways. In bacteria this may occur via an NAD-(in)dependent formaldehyde dehydrogenase or (in serine-pathway organisms, see below) via enzymes of the methenyltetrahydrofolate pathway. The formate produced is finally converted into CO_2 via NAD-dependent formate dehydrogenase. Another possible mechanism for complete oxidation of formaldehyde into CO_2, present only in methane non-utilizing bacteria with the RuMP cycle (see below), involves a cyclic sequence of reactions in which enzymes of the oxidative pentose-phosphate pathway (glucose-6-phosphate dehydrogenase/6-phosphogluconate dehydrogenase) play an important role. This pathway does not require formaldehyde and formate dehydrogenase activities. In yeasts, an NAD/glutathione-dependent formaldehyde dehydrogenase is exclusively involved in formaldehyde oxidation. The product of this reaction, *S*-formylglutathione, is further metabolized to CO_2 via either a hydrolase and an NAD-dependent formate dehydrogenase, or directly via the latter enzyme.

In aerobic methylotrophs carbon assimilation proceeds initially by conversion of three C_1 units into a C_3 compound via a cyclic pathway. In bacteria three of these pathways are now firmly established, namely the ribulosebisphosphate (RuBP) or Calvin cycle (e.g. in *Paracoccus denitrificans, Xanthobacter autotrophicus*), the ribulosemonophosphate (RuMP) cycle (e.g. in *Pseudomonas methanica, Methylophilus methylotrophus*), Fig. 3, and the serine pathway (e.g. in *Pseudomonas* AM1; *Hyphomicrobium* X), Fig. 4. The assimilation pathway in yeasts, the dihydroxyacetone (DHA) or xylulosemonophosphate (XuMP) cycle, Fig. 3, has only recently been elucidated.

The Calvin cycle in methylotrophs

It is clear from Fig. 3 that the three pathways involving sugar phosphate molecules as intermediates are very similar in design. The main differences are found in the identity of the enzymes specifically involved in the initial reactions by which the C_1 units are fixed, namely ribulosebiphosphate carboxylase/phosphoribulokinase (RuBP cycle), hexulosephosphate synthase/hexulosephosphate isomerase (RuMP cycle) and dihydroxyacetone synthase/dihydroxyacetone kinase (XuMP cycle). Further steps in these cycles are catalysed by enzymes from the glycolytic or Entner–Doudoroff pathways (i.e. fructosebisphosphate aldolase, FBPA, or 2-keto-3-desoxy-6-phosphogluconate aldolase, KDPGA, respectively) and the oxidative pentosephosphate pathway involving either sedoheptulosebisphosphatase (SBPase) or transaldolase (TA). Based on the various possible combinations of these enzyme systems, three variants of the RuMP cycle have been encountered in different methylotrophic bacteria (Table 1).

The serine pathway

Intermediates of the serine pathway are mainly organic acids and amino acids. Enzymes specifically involved in this pathway are serine transhydroxymethylase, serine:glyoxylate aminotransferase and hydroxypyruvate reductase. Further steps in this pathway are catalysed by enzymes of the citric acid cycle. In most serine pathway organisms the glyoxylate cycle enzyme, isocitrate lyase, is absent and the identity of the enzymes involved in the regeneration of glyoxylate remains to be established.

Table 1. Metabolic energy required for the conversion of C_1-units into C_3 compounds

Cycle	*C_1 units fixed*	*Primary product*	*Variant*	*ATP required*	*$NAD(P)H_2$ required*
RuBP	$3CO_2$	PGA	SBPase	8	5
RuMP	3HCO	GAP	FBPA/TA	1	0
		GAP	FBPA/SBPase	2	0
		pyruvate	KDPGA/TA	0	−1
serine	2HCHO + $1CO_2$	PGA	icl^+	3	2
XuMP	3HCO	GAP	SBPase	3	0

For abbreviations: see legend Fig. 3, and SBPase: sedoheptulosebisphosphates; FBPA: fructosebisphosphate aldolase; TA: transaldolase; KDPGA: 2-keto-3-desoxy-6-phosphogluconate aldolase; icl^+: isocitrate lyase positive.

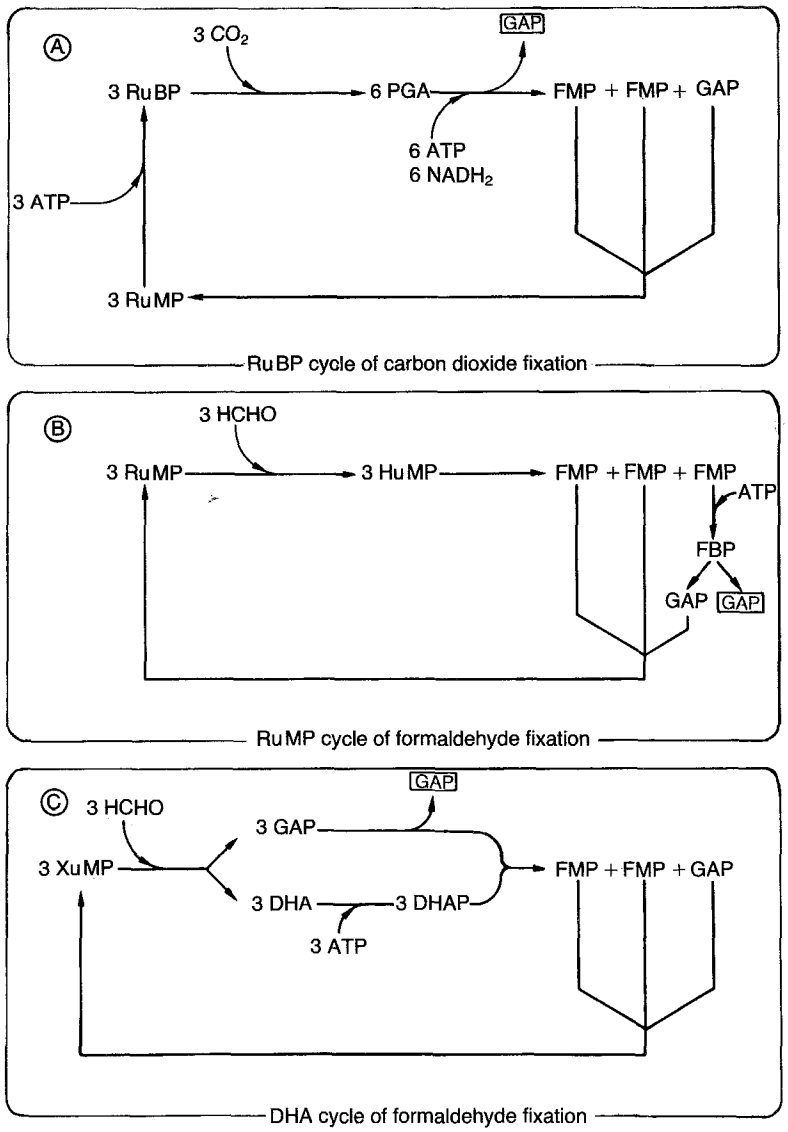

Fig. 3. Schematic representation of the Calvin cycle (A), the ribulosemonophosphate cycle (B) and the dihydroxyacetone or xylulosemonophosphate cycle (C). RuBP: ribulose-1,5-bisphosphate; RuMP: ribulose-5-phosphate; PGA: 3-phosphoglycerate; GAP: glyceraldehyde-3-phosphate; FMP: fructose-6-phosphate; HuMP: 3-hexulose-6-phosphate; FBP: fructose-1,6-bisphosphate; XuMP: xylulose-5-phosphate; DHA: dihydroxyacetone; DHAP: dihydroxyacetone phosphate.

Depending on the type of assimilation pathway, a large variation can be observed in the metabolic energy required for synthesis of the primary C_3 output molecule. This is also summarized in Table 1 and the data show that the RuMP cycle is energetically the most efficient, followed by the XuMP cycle in yeasts. These differences are reflected in the experimental growth yields observed in organisms growing on methanol, and range from around 0.55 g dry weight (g methanol)$^{-1}$ in RuMP cycle organisms to 0.37–0.45 g g^{-1} in serine pathway organisms and yeasts. The fact that the growth yield in the latter organisms is in the same range as for bacteria using the serine pathway is due to the involvement of an oxidase in the conversion of methanol to formaldehyde.

Anaerobic methanol-utilizing bacteria

Obligately anaerobic methanol-using bacteria are found among the methanogenic and the acidogenic bacteria. Methanogenic bacteria such as *Methanosarcina barkeri* convert methanol to CH_4 and CO_2 (for equations see Table 2); their growth is slow and molar growth yields on methanol are low. Acidogenic bacteria that grow on methanol were first described in 1980 (*Butyribacterium methylotrophicum*); six additional species have since been shown to grow on methanol with the formation of organic acids as major fermentation products (Table 3). Some of these bacteria have been known for several years but their methylotrophic potential had remained undiscovered. Methylotrophic acidogens have been the subject of some excellent recent reviews[3,16,17]. Most methylotrophic acidogenic bacteria can also use other C_1-compounds such as H_2 + CO_2 and carbon monoxide. Betaine and choline also function as C_1-substrates for strains of *Eubacterium* and *limosum*, but in this process only one of the methyl groups is utilized and this results in the formation of *N,N*-dimethylglycine and *N,N*-dimethylethanolamine, respectively. Similarly, growth of *Acetobacterium woodii* on several methoxylated aromatic compounds is, in fact, methylotrophic since only the methoxy groups are used. None of the methanol-using acidogens, however, is metabolically restricted to C_1-substrates for energy generation; all grow on a variety of other substrates including some sugars. Most of the acidogens form acetate from methanol (reaction b in Table 2). *Butyribacterium methylotrophicum*, however, forms butyrate as the main product and so does *Eubacterium limosum*. The latter species forms small amounts of caproate in addition. During growth of both species on H_2 + CO_2, or on CO, acetate is by far the major product.

Organisms such as *Butyribacterium methylotrophicum* have been shown to grow in minimal media supplemented with vitamins[18] so that they can derive virtually all of their cell carbon from C_1-substrates. The most likely path-

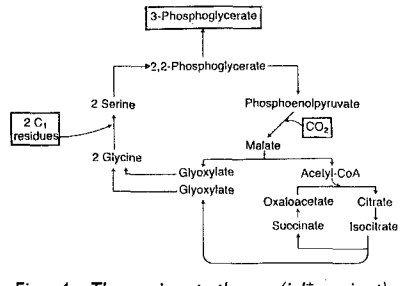

Fig. 4. The serine pathway (icl$^+$-variant). Reproduced from Ref. 14 with permission.

way of cell carbon synthesis is via acetyl-CoA. Studies on the biochemical pathway of the homoacetate fermentation have mainly been performed with *Clostridium thermoaceticum* as a model organism. This organism is usually grown on sugars and forms three moles of acetate per mole of the C_6-sugar utilized. Growth on H_2 + CO_2 and on CO, but not on methanol, has been documented. The pathway of acetate formation in *Clostridium thermoaceticum* is shown in Fig. 5. Although enzyme measurements and ^{13}C NMR studies[19] performed with *Acetobacterium woodii, Clostridium thermoautotrophicum* and *Butyribacterium methylotrophicum* support the outline of the mechanism of acetate formation as depicted in Fig. 5, several details of this scheme remain to be elucidated. A central role is played by carbon monoxide dehydrogenase.

This enzyme was purified from *Clostridium thermoaceticum* and *Acetobacterium woodii* and shown to contain iron-sulfur clusters and nickel. It is thought to be involved in the reduction of CO_2 to an intermediate at the level of CO, and in the activation of CO. The methyl group of acetate is formed from CO_2 via C_1-tetrahydrofolates during growth on CO_2 + H_2 or CO. In the initial step of this series of reactions a formate dehydrogenase is involved which may also function as a CO_2 reductase. The detailed mechanism of the formation of the acetyl-group from CH_3-tetrahydrofolate and CO (or CO_2 and H_2) is being studied; rapid progress is to be expected, however, since Hu *et al.*[20] reported the synthesis of acetyl-CoA from CO, CH_3-tetrahydrofolate, CoA and ATP in the presence of a purified methyltransferase, CO dehydrogenase, a purified corrinoid protein and an enzyme X with a hitherto unknown function. Recently[21], evidence was presented for the view that CO dehydrogenase is the condensing enzyme that forms acetyl-CoA from its component parts.

Table 2. Some reactions involving anaerobic conversions of C_1-compounds

Reaction	$\Delta G_0'$ *kJ*
(a) $4CH_3OH \rightarrow 3CH_4 + HCO_3^- + H^+ + H_2$	−314.8
(b) $4CH_3OH + 2HCO_3^- \rightarrow 3acetate^- + H^+ + 4H_2O$	−221.8
(c) $4CH_3OH + 0.8\,HCO_3^- \rightarrow 1.2butyrate^- + 0.4H^+ + 4H_2O$	−216.7
(d) $4H_2 + H^+ + 2\,HCO_3^- \rightarrow acetate^- + 4H_2O$	−104.6
(e) $4CO + 4H_2O \rightarrow acetate^- + 2HCO_3^- + 3H^+$	−165.4

It remains to be established exactly how methanol enters the pathway to acetate (or butyrate). In *Eubacterium limosum,* methanol metabolism is initiated by a reaction with a methyltransferase (MT1) which contains firmly bound cobalamin[22]. Formation of butyrate should probably be regarded as a variant of acetate formation in that acetyl-CoA is reduced to butyrate.

Methylotrophs and biotechnology

In industrial fermentations the price of the feedstock is of crucial importance in determining the economic viability of the process. In view of the low cost of methanol and its availability in large amounts in a very pure form, it is an attractive alternative substrate. The possession of unique sets of enzymes enables methylotrophic microorganisms to convert methanol into compounds containing carbon–carbon bonds. The products of these primary carbon assimilation reactions can subsequently be used in further biosynthetic pathways so that all the monomers and polymers required for cell growth and multiplication can be synthesized from the C_1 substrate.

For an aerobic methylotrophic bacterium using the FBPA/TA variant of the RuMP cycle it can be calculated that the conversion of methanol into a hexosemonophosphate occurs according to the following stoichiometry:

6 methanol + 6 PQQ + 2 ATP → glucose 6-phosphate + 6 $PQQH_2$ + 2 ADP + 2P_i.

The 6 moles of methanol and one mole of glucose, approximately equivalent on a weight to weight basis, are also very similar bioenergetically. A major problem of using these aerobic, methylotrophic organisms in industrial processes, however, is their high oxygen demand since the reduction equivalent ($PQQH_2$) produced in the above reaction must be reoxidized. In this respect the anaerobic, acidogenic methylotrophic bacteria offer an attractive alternative.

Despite the feasibility and the economic attraction of using methanol as a replacement feedstock in industrial fermentations, potential processes should be selected carefully. First of all it is clear that, for established processes, a change to methanol involving selecting and breeding a methylotroph as a novel production strain, can only be considered if the cost of the fermentation broth is relatively high compared to the value of the product. Thus, research on potential biotechnological applications based on methanol as a cheap feedstock should be aimed mainly towards the production of bulk chemicals and not towards high added-value products such as hormones and proteins of medical or veterinary importance. A second reason for considering methanol as a feedstock may be that using a methylotroph opens up new metabolic routes for the synthesis of various products. Thirdly, an advantage may be gained from the unique properties and metabolism of methylotrophs thereby exploiting their potential use as vehicles for the expression of heterologous genes, as

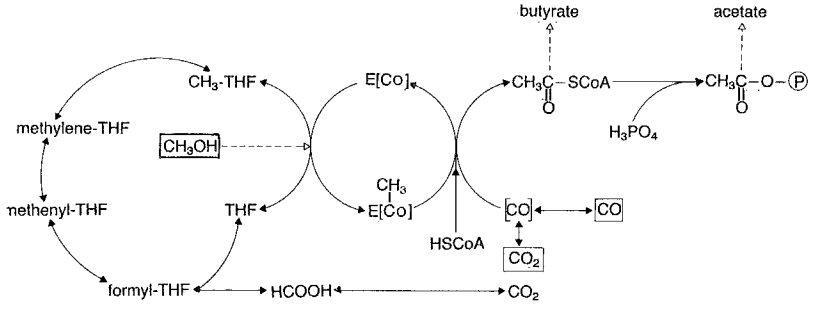

Fig. 5. Pathway of acetate and butyrate formation in anaerobic, acidogenic bacteria growing on C_1-substrates. THF: tetrahydrofolate; E[Co]: corrinoid protein.

Table 3. Anaerobic methanol-using acidogenic bacteria

Species	Main or sole fermentation product from methanol
Acetobacterium woodii	acetate
Clostridium formicoaceticum	acetate
Clostridium thermoautotrophicum	acetate
Butyribacterium methylotrophicum	butyrate
Eubacterium limosum	butyrate
Sporomusa ovata	acetate
Sporomusa sphaeroides	acetate

novel biocatalysts and in the production of metabolites.

Practical application of methylotrophs

Judging from the large number of patents covering many presumptive processes involving methylotrophs[23] there is currently wide interest in this area of biotechnological research (Table 4). However, to our knowledge only a limited number of processes has been realized. Early on the main emphasis was on the production of single-cell protein using bacteria (i.e. *Methylophilus methylotrophus, Methylomonas clara*) or yeasts (*Pichia pastoris*) growing aerobically on methanol. The various parameters of importance in these processes have been studied extensively[5,7,8] and at the moment plants are operating in a number of countries. The economics of these processes, however, are strongly dependent on the fluctuations in the price of alternative protein sources and suffer from the restricted market for SCP. Interest is now turning more and more towards the production of metabolites and primary products, such as polysaccharides, amino acids, vitamins and coenzymes[24]. Research in this direction is still mainly at the stage of selection of suitable strains and their further improvement via conventional breeding techniques.

It can be predicted that the greatest advantage of methanol as a feedstock will be in those processes which aim to use specific properties of methanol metabolism. This is reflected in the current interest in the production of serine using serine pathway organisms and of aromatic amino acids (via erythrose-4-phosphate and PEP) with RuMP cycle methylotrophs. With respect to vitamins and coenzymes the most promising prospects appear to be riboflavin and vitamin B_{12}, both of which are synthesized at high rates under certain growth conditions in yeasts (riboflavin) and both anaerobic and aerobic bacteria (vitamin B_{12}), respectively. Furthermore, the possible application of the novel biocatalysts encountered in methylotrophs, in enzyme electrodes, fuel cells and for cofactor regeneration, are being investigated extensively.

Finally use can be made of other specific properties of methylotrophs. For example, under certain conditions alcohol oxidase constitutes up to 30% of the total soluble protein in peroxisomes of methylotrophic yeasts. The potential of these organisms as vehicles for expressing foreign genes and for packaging foreign proteins in subcellular organelles where they are protected against proteolysis is currently being investigated.

Anaerobic methylotrophic bacteria

Given the high oxygen demand of aerobic methylotrophic bacteria, the anaerobic, acidogenic methylotrophic bacteria might be thought to be more suitable for the production of organic acids such as acetate and butyrate, the end-products of the fermentative processes in these organisms. Acetate, however, is already produced efficiently on a large scale from methanol and CO by the chemical industry[1] and it seems unlikely that a biotechnological process could become competitive.

Table 4. Potential use of methylotrophs in bioprocesses

Single-cell protein	enzyme electrodes
Polysaccharides	fuel cells
Amino acids	cofactor regeneration
Vitamins	coenzymes

Similarly, while butyrate can easily be esterified (the possible uses of methyl and ethyl esters of butyrate have been discussed by Zeikus[3]) significant advances in the fermentation of methanol to butyrate and in separation processes would be needed before biotechnological butyrate production could emerge as a commercial success[3,25,26]. Such constraints might also apply to the production of propionate. Reduction of acetate + CO_2 by hydrogen to propionate is catalysed by *Desulfobulbus propionicus*[27]; mixed cultures or two-stage cultures of an acetogenic methanol-fermenting bacterium and *Desulfobulbus*, therefore, offer, at least theoretically, a way of producing propionate from methanol.

The anaerobic, acidogenic methylotrophic bacteria might provide a source of other commercially interesting compounds, however. Up to 28% of the methanol carbon is recovered in cell carbon during growth of *Butyribacterium methylotrophicum* on methanol, a value which is considerably lower than during growth of aerobes on methanol (see above). This is not unexpected in view of the far smaller energy gain in acidogenesis as compared with oxidation of methanol to CO_2. The anabolic process requiring most ATP is protein synthesis. The synthesis of central intermediates of carbon metabolism from methanol and CO_2, however, can be carried out with only modest investments of ATP and, therefore, production in reasonable yields of amino acids, for instance, should in principle also be possible with anaerobes. Acidogenic methylotrophs have been known for only five years. It is therefore not surprising that so far relatively few studies have dealt with possible applications of these microbes.

Conclusions

Recent advances in our understanding of the physiology and biochemistry of methylotrophs have made it possible, at least in principle, to evaluate their potential in biotechnological processes. The range of organisms with interesting and promising properties is continuously increasing and it is to be expected that they will find specific applications in bioprocesses based on methanol as a feedstock. Although the metabolic pathways of methanol metabolism are largely known, our current level of understanding of the regulation of metabolic fluxes in methylotrophs, particularly those leading to useful products, is less well developed. This is currently the main focal point of research and one which is essential for the development of future biotechnological processes based on methanol.

References

1 Swift, H. E. (1983) *Am. Sci.* 71, 616–620
2 Higgins, I. J., Best, D. J. and Scott, D.

(1982) in *Genetic Engineering of Microorganisms for Chemicals* (Hollaender, A., DeMoss, R. D., Kaplan, S., Konisky, J., Savage, D. and Wolfe, R. S., eds) *Basic Life Sciences*, Vol. 19, pp. 383–402, Plenum Press
3 Zeikus, J. G. (1983) in *Organic Chemicals from Biomass* (Wise, D. L., ed.), pp. 359–383, The Benjamin Cummings Publishing Company
4 Daniels, L. (1984) *Trends Biotechnol.* 2, 91–98
5 Anthony, C. (1982) *The Biochemistry of Methylotrophs*, Academic Press
6 Large, P. J. (1983) *Methylotrophy and Methanogenesis. Aspects of Microbiology*, Vol. 8, Van Nostrand Reinhold
7 Hou, C. T. (ed.) (1984) *Methylotrophs: Microbiology, Biochemistry, and Genetics*, CRC Press Inc.
8 Dalton, H. (ed.) (1981) *Microbial Growth on C_1 Compounds*, Heyden
9 Crawford, R. L. and Hanson, R. S. (eds) (1984) *Microbial Growth on C_1 Compounds*, American Society for Microbiology, Washington D.C.
10 Colby, J., Dalton, H. and Whittenbury, R. (1979) *Annu. Rev. Microbiol.* 33, 481–517
11 Quayle, J. R. and Ferenci, T. (1978) *Microbiol. Rev.* 42, 251–273
12 Quayle, J. R. (1980) *Biochem. Soc. Trans.* 8, 1–10
13 Quayle, J. R. (1980) *FEBS Lett.* 117, K16–K27
14 Harder, W. and Attwood, M. M. (1978) *Adv. Microb. Physiol.* 17, 303–359
15 Veenhuis, M., van Dijken, J. P. and Harder, W. (1983) *Adv. Microb. Physiol.* 24, 1–78
16 Zeikus, J. G. (1983) *Adv. Microb. Physiol.* 24, 213–299
17 Ljungdahl, L. G. (1983) in *Organic Chemicals from Biomass* (Wise, D. L., ed.), pp. 219–248, The Benjamin Cummings Publishing Company Inc.
18 Moench, T. T. and Zeikus, J. G. (1983) *Curr. Microbiol.* 9, 151–154
19 Kerby, R., Niemczura, W. and Zeikus, J. G. (1983) *J. Bacteriol.* 155, 1208–1218
20 Hu, S. I., Pezacka, E. and Wood, H. G. (1984) *J. Biol. Chem.* 259, 8892–8897
21 Ragsdale, S. W. and Wood, H. J. (1985) *J. Biol. Chem.* 260, 3970–3977
22 van der Meijden, P., van der Drift, C. and Vogels, G. D. (1984) *Arch. Microbiol.* 138, 360–364
23 Hou, C. T. (1984) in *Methylotrophs: Microbiology, Biochemistry and Genetics* (Hou, C. T., ed.), pp. 145–166, CRC Press
24 Kato, N., Tani, Y. and Yamada, H. (1983) in *Advances in Biotechnological Processes* (Mizrahi, A. and van Wezel, A. L., eds), Vol. 1, pp. 171–202, Alan R. Liss
25 Datta, R. (1981) *Biotechnol. Bioeng. Symp.* 12, 177–182
26 Datta, R. and Ogeltree, J. (1983) *Biotechnol. Bioeng.* 25, 991–998
27 Laanbroek, H. J., Abee, T. and Voogd, I. L. (1982) *Arch. Microbiol.* 133, 178–184

Plasma protein fractionation

E. R. A. Jeans, P. J. Marshall and C. R. Lowe

The fractionation of blood plasma yields a number of therapeutic proteins which together constitute a billion dollar world-wide market. Conventional plasma fractionation procedures are being replaced by more sophisticated chromatographic techniques. The application of genetic engineering and these new downstream processing techniques is likely to lead to improved products of higher purity and stability, with less likelihood of contamination by viruses and at lower cost per therapeutic dose.

Whole blood plasma contains numerous proteins of potential therapeutic, and hence, economic value. Separating or fractionating these plasma proteins not only enables the treatment of several patients using any given blood sample but also upgrades the potency of the resulting proteins to many times that of whole plasma. For example, a factor VIII concentrate, which contains at least 15-times the normal plasma concentration of clotting factor VIII, is routinely used in the control of bleeding in haemophiliacs.

Most of the amenable clinical conditions are treated by replacement with the appropriate plasma protein concentrate either as a prophylactic or after injury. For example, albumin is widely used as a blood-volume expander, after blood loss as a result of surgery, accident or post-partum haemorrhage. Similarly, the most commonly used immunoglobulins are anti-Rh(D) as a prophylactic for Rh allo-immunization in pregnancy, anti-tetanus and anti-rubella[1]. The physiological and clinical properties of these and other proteins of current commercial interest are summarized in Table 1.

E. R. A. Jeans, P. J. Marshall and C. R. Lowe are at The Biotechnology Centre, University of Cambridge, Downing Street, Cambridge CB2 3EF, UK.

World market

Economically, albumin and factor VIII are the most important plasma proteins, with world market values ranging from \$250 million–750 million for albumin and \$200 million–275 million for factor VIII. Immunoglobulins are used extensively in some countries such as Germany and Japan, and represent \$300 million–500 million on the world market[6]. Albumin, which accounts for about 38% of the total world market in plasma proteins, sells at \$2.50 per gram with an average dose of 12.5 g costing \$30–35. Factor VIII represents 18% of the market and, if it were available as a homogeneous preparation, would be considerably more expensive at \$500 000 per gram (7–14 US cents/unit of activity). An average dose of 250 units contains only 12.5 μg of factor VIII protein and costs \$17–35. In Europe, however, factor VIII is more expensive at 30 US cents/unit or \$75 per 250 unit dose. The largest collector, processor and exporter of plasma and plasma-derived products is the USA. According to the American Blood Resources Association[6], of the 9.5×10^6 litres of plasma consumed annually world-wide, the USA collects a total of 7.7×10^6 litres via volunteers and the plasma industry.

The USA, Germany and Japan are the major consumers of plasma and plasma-derived products, between them consuming 65% of the total world market in 1980, which was then estimated at \$1.2 billion; on the assumption that the market increases by 10% a year, it could now be worth a staggering \$1.75 billion.

Cohn fractionation

In 1946, a process was devised for the fractionation of plasma proteins[7]. The Cohn procedure was based on the differential precipitation of the various proteins by appropriate combinations of ethanol concentration, low temperatures, ionic strength, pH and total protein concentration. The conditions used for obtaining the Cohn fractions and their constituents are summarized in Table 2. Over the years, numerous improvements have been made to the Cohn procedure to enhance the economy of production and purity of the relatively few proteins used in large quantities in clinical practice, i.e. albumin, immunoglobulins and factor VIII. Not surprisingly, therefore, most